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RESEARCH ARTICLE

Standardization of Spawn and Substrate Formulations for *Morchella* Mushroom Cultivation

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A B S T R A C T

The present study evaluated various cultural media and substrate formulations to optimize the cultivation of *Morchella esculenta*. Three culture media, Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Czapek-Dox Agar (CDA), were tested for mycelial growth at 25°C over 3, 5, and 7 days. PDA proved most effective, supporting the fastest and most extensive mycelial expansion (8.4 cm by day 7), followed by MEA (7.2 cm) and CDA (5.4 cm), likely due to its nutrient-rich composition. For spawn production, three cereal grains, barley, wheat, and white sorghum, were assessed over 7, 14, and 21 days. Barley supported the most vigorous growth (9.0 cm by day 21), followed by wheat (8.0 cm) and sorghum (6.0 cm), suggesting barley offers optimal nutrition and texture for spawn development. Substrate evaluations without nutrient bags involved three formulations: Compost-Potting Soil-Sand, Hardwood Chips, and Sawdust. While all supported sclerotia and pinhead formation, none produced mature fruiting bodies. Hardwood Chips showed the best early development (5 fruiting bodies), yet nutrient limitations restricted full maturation. With nutrient bags, substrate performance improved. Soil + Wheat Straw yielded 20 fruiting bodies with the fastest development. Soil + Organic Matter + Peat Moss produced the highest number of fruiting bodies (22), though no mature mushrooms were harvested. The enhanced initiation stage with nutrient supplementation highlights its importance, but the absence of full maturation across treatments suggests environmental factors or micronutrient deficiencies may be limiting. Further refinement of growth conditions and nutrient inputs is necessary for successful *Morchella* cultivation.

Keywords: *Morchella*, Morel, Spawn, Compost, Cultivation, Standardization, Mycelium.

INTRODUCTION

Morels, commonly known as *Morchella*, are highly prized edible mushrooms renowned for their distinctive honeycomb-like appearance and rich flavor. According to Wu *et al*. (2021), morels are closely related to cup fungi of the order *Pezizales* and belong to the phylum *Ascomycota* (sac fungi). Morel harvesting is popular for recreational, culinary, and commercial purposes, with these mushrooms being especially valued in gourmet cuisine (Du *et al*., 2015). The most well-known types of morels are the semi-free morel (*Morchella semilibera*), yellow morel (*M. esculenta*), and black morel (*M. elata*) (Bunyard *et al*., 1995; Du, 2019; Du *et al*., 2020). Due to their ecological and economic

significance, *Morchella* species have become a focal point of scientific research and conservation efforts aimed at promoting sustainable use (Li *et al.,* 2013).

Morels are native to various continents, including Europe, North America, Australia, Asia, and New Zealand. They typically grow in meadows, forests, and woodlands with nutrient-rich soil and mild temperatures. Many species form mycorrhizal associations, a symbiotic relationship with tree roots, particularly those of elm, oak, and ash (Kapoor *et al*., 2024). Some morels also appear in areas affected by wildfires. Even though morels are highly sought after for their flavor, correct identification is crucial, as some *Morchella* species are toxic or inedible (Vieira *et al*., 2016).

Morels can be found in diverse environments, including urban areas, and usually emerge in early spring. While foraging is common, commercial cultivation is also practiced. Despite widespread interest, the biology and life cycle of morels remain complex and are the subject of ongoing research. For instance, Liu *et al*. (2018a) studied yellow morels (*Esculenta* clade) and described the formation of sclerotia, nutrient-storing, weather-resistant structures that can either develop into fruiting bodies or revert to mycelium depending on environmental conditions. Liu *et al*. (2018a) reported that some black morel species (*Elata* clade) in western North America reproduce asexually via mitospores, though the role of this process in the overall life cycle is not fully understood.

As mycorrhizal fungi, morels engage in mutually beneficial relationships with certain trees, exchanging nutrients for sugars and carbohydrates. Under optimal conditions, they produce fruiting bodies when soil temperatures rise above 50-60°F (10-16°C) in the spring (Masaphy *et al*., 2010).

Artificial cultivation of morels is possible using growth media that mimic natural conditions, such as compost, sawdust, and nutrient-rich substrates. Spores obtained from reliable sources can be introduced into these media. Another method involves using mycorrhizal seedlings of specific trees planted in enriched soil to encourage morel development (Liu *et al*., 2018b; Khan *et al*., 2024).

China has become a global leader in morel production, particularly through the use of nutrient bags to promote mycelial growth. Successful large-scale cultivation of black morels such as *M. sextelata*, *M. importuna*, and *M. septimelata* has been reported. Research by Penn State's Siyi Ge has identified optimal conditions for growing *M. importuna* and *M. rufobrunnea*, including ideal light, temperature, and pH levels. These findings suggest potential for outdoor cultivation in northeastern U.S. regions (Liu *et al*., 2018a).

Efforts are also underway to optimize indoor cultivation, allowing for year-round production with controlled humidity, temperature, and ventilation. Ower's (1986) method, later adapted for commercial use, emphasizes the importance of sclerotia formation and has influenced modern cultivation techniques (Pilz *et al*., 2004; Masaphy, 2010).

Despite progress, yield variability remains a significant challenge. Many growers face low or inconsistent production due to limited understanding of morel biology, mating systems, and spawn quality. The present study aims to address these issues by standardizing spawn production and evaluating effective substrates for *Morchella* cultivation.

MATERIALS AND METHODS

Sample Collection: The *M. rufobrunnea* culture (MMFL231) was obtained from the Fungal Plant Pathology Laboratory, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan, for further study.

Culture Media for *Morchella* Mushroom: Three culture media, Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Czapek Dox Agar, were used to determine the optimal medium for *Morchella* mycelial growth. Petri dishes were sterilized in an autoclave at 121°C for 60 minutes. After sterilization, the media were poured into the plates and allowed to cool (Atiq *et al*., 2022; Usman *et al*., 2024; Yaqoob *et al*., 2024).

To initiate tissue culture, the ascocarp of the *Morchella* mushroom was carefully cut using a sterile needle, and tissue from the stipe region was aseptically excised. Small pieces from the core were placed on the prepared media plates, which were sealed with parafilm and incubated at 25°C. Mycelial growth appeared within a week. PDA and MEA were found to be the most effective media for promoting *Morchella* mycelial growth (Kalm and Kalyonce, 2008).

Spawn Preparation: Three grain types, white sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*), were used for spawn preparation. Half a kilogram of each grain was cleaned manually to remove debris and soaked in water overnight. The following day, the grains were washed again and boiled for 10-15 minutes until soft but intact. Excess water was drained, and the grains were cooled.

To maintain a loose texture and favorable pH, 20 g of calcium carbonate and 1% magnesium sulfate were added

per 500 g of grain. The grains were then packed into polythene bags or clean jars, leaving one-third of the space empty. These containers were sealed with non-absorbent cotton and autoclaved at 121°C for 60 minutes at 15 psi.

After cooling, the jars were transferred to a sterile inoculation chamber. Mature mother spawn (18-20 days old) was broken into small pieces using sterile forceps and inoculated into the sterilized grain jars. The jars were resealed and incubated at 13°C-18°C. To ensure even colonization, jars were shaken daily. Contaminated jars were discarded. After 15-20 days, the grains were fully colonized with mycelium and ready for use (Siddhant *et al*., 2021).

Evaluation of substrate treatments for *Morchella* cultivation without nutrient bag: To evaluate different substrate treatments for *Morchella* cultivation without nutrient bags, compost was evenly spread to a depth of two inches in trays and thoroughly moistened using a sprinkler. Excess water was drained, and limestone was added to adjust the pH. The *Morchella* spawn was then evenly spread over the surface.

Trays were incubated in darkness at 18-20°C with 90% humidity for 4-6 weeks. Sclerotia, dense, seed-like structures of hardened mycelium, developed across the substrate surface. After full colonization, trays were refrigerated at 3-4°C for several weeks, then transferred back to 18°C with 90% humidity and natural light.

Substrates were misted twice daily. When primordia (tiny mushroom caps) began to form, the humidity was gradually reduced to around 60%, and the temperature was increased to 21.7-22.7°C. Mushrooms were exposed to 12 hours of light daily. Fully developed mushrooms were harvested by cutting at the base, allowing smaller mushrooms and stems to continue growing. Different substrate formulations were evaluated, including:

1. 50% organic compost, 30% potting soil, 20% sand (Adams *et al*., 2022)
2. 10% rice hulls, 5% peat moss, 5% soybean meal, 80% hardwood chips
3. 10% rice hulls, 5% peat moss, 5% soybean meal, 80% sawdust (Wang *et al*., 2015)

Evaluation of substrate composition for Morchella cultivation with nutrient bags: To evaluate substrate composition with nutrient bags, the substrate was sterilized in an autoclave for 1 hour at 121°C and 15 psi. The mixture was poured into plastic tubs (45 cm × 60 cm × 18 cm), each fitted with three holes (2 cm diameter) on one side to facilitate drainage.

Seventy-two hours before spawning, 23 kg of soil was added to each tub. Spawning occupied roughly 25% of the tub surface. After rinsing, tubs were covered with black plastic sheets and maintained at 16°C.

Nutrient bags were prepared either one day before use or eight days after spawning. Each 350 g bag contained:

1. 209.6 g of corn/wheat (wet weight, ~5.5–7% moisture)
2. 140.6 g sawdust (wet weight, ~8% moisture)
3. 157.4 g of water (to maintain ~55% total moisture)

The bags were sterilized twice at 121°C for 40 minutes and allowed to cool overnight. Seven days after spawning, two nutrient bags were cut open lengthwise (~19 cm) and placed 3 cm apart in the center of each tub under a plastic sheet.

Forty-nine days after spawning, the plastic covers were removed, and lighting was introduced to simulate day-night cycles (12 hours on, 12 hours off). Nutrient bags were removed to promote fruiting. Mature fruiting bodies were harvested, counted, and weighed between 100 and 124 days after spawning.

Substrate combinations evaluated included:

1. 50% soil + 50% wheat straw
2. 50% soil + 50% sawdust
3. 50% soil + 25% organic matter + 25% peat moss (Tian *et al*., 2020)

STATISTICAL ANALYSIS

Data were analyzed using Statistix 8.1 software. A Completely Randomized Design (CRD) was used for the experiment. Treatment means were compared using a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The present study evaluated multiple cultural media, substrates, and substrate treatments (with and without nutrient supplementation) to determine optimal conditions for the mycelial growth and fruiting of *M. esculenta*. Results clearly showed significant variation in the efficiency of different media and substrates in supporting vegetative and reproductive growth phases of this high-value edible fungus.

Mycelial Growth on Culture Media: Among the three tested culture media, PDA, MEA, and CDA, PDA supported the most rapid and extensive mycelial growth of *M. esculenta* (Table 1), consistent with previous findings (Chutia and Ahmed, 2012; Kumawat *et al*., 2016; Castillo *et al*., 2017; Hassan *et al*., 2023; Abouzkhar *et al*., 2024; Pham *et al*., 2024). By the 7th day of incubation, PDA supported growth of 8.4 cm, outperforming MEA (7.2 cm) and CDA (5.4 cm). PDA's superiority may be attributed to its high

carbohydrate content and balanced pH, which foster optimal conditions for fungal metabolism and enzyme activity (Wu *et al*., 2008; Kim *et al*., 2019; Iqbal *et al*., 2021; Lane, 2023). Mycological studies have long documented PDA's effectiveness in promoting the vegetative phase of various fungal species due to its rich nutrient profile (Rabbani *et al*., 2011; Sia *et al*., 2013; Wongjiratthiti and Yottakot, 2017; Westphal *et al*., 2021; Alasadi, 2024).

Table 1. Evaluation of different media for mycelial growth.

Days Interval	Use of Different Media for Mycelial Growth of *Morchella* (cm)		
	Potato Dextrose Media (PDA)	Malt Extract Agar (MEA)	Czapek Dox Agar Media (CDA)
Day 03	4.3 A	3.2 B	1.4 C
Day 05	7.2 A	6.4 B	3.2 C
Day 07	8.4 A	7.2b	5.4c

Spawn Growth on Different Cereal Grains: Barley proved to be the most effective cereal grain for spawn production, achieving full colonization (9.0 cm) by day 21 (Table 2). This aligns with the work of Girmay *et al.* (2016), who reported that barley supports rapid colonization in several edible fungi due to its high starch content and favorable texture. Wheat also supported substantial growth (8.0 cm), affirming its role as a commonly used, effective substrate in mushroom cultivation (Pathmashini *et al*., 2009). In contrast, white sorghum lagged significantly in supporting mycelial spread, which may be due to lower nutrient availability or poor aeration characteristics (Julian *et al*., 2020; Vargas-Solórzano *et al*., 2014). These differences underscore the importance of substrate selection in optimizing spawn vigor.

Table 2. Evaluation of substrates for spawn preparation.

Day's interval	Substrates for Morchella (cm)		
	Barley Grain	Wheat Grain	White Sorghum
7	4.0 c	3.5 c	2.5 c
14	7.5 ab	6.2 b	4.5 ab
21	9.0 a	8.2 a	6.0 a

Substrate Treatments without Nutrient Bags: All three substrate treatments, Compost-Potting Soil-Sand, Hardwood Chips, and Sawdust, supported initial colonization and pinhead formation but failed to produce mature fruiting bodies (Table 3). The limited fruiting success may be due to inadequate nutrient availability in the absence of supplemental feeding. Similar observations were reported by Masaphy (2010), who emphasized that *Morchella* species require nutrient-rich environments and controlled environmental parameters to achieve complete fruiting. While hardwood chips yielded more fruiting bodies (n = 5), they still failed to mature, indicating that initial aeration and carbon sources alone are insufficient for full development (Singh *et al*., 2011). This supports the hypothesis that additional supplements or environmental manipulations are essential for reproductive success in *Morchella* cultivation.

Table 3. Evaluation of different compost substrates for mushroom cultivation.

Treatments	Spawn Run (days)	Pinhead Formation (days)	No. of Fruiting Bodies	Total Yield (g)
50% organic compost, 30% potting soil, 20% sand	25 a	35 a	3 b	2 b
10% rice hulls, 5% peat moss, 5% soybean meal, 80% hardwood chips	22 b	32 b	4 b	3.5 a
10% rice hulls, 5% peat moss, 5% soybean meal, 80% sawdust	24 a	34 a	7 a	4.3 a

Substrate Treatments with Nutrient Bags: The inclusion of nutrient bags significantly improved the initiation of reproductive structures in all treatments. The Soil + Organic Matter + Peat Moss treatment produced the highest number of fruiting bodies (n = 22), suggesting that organic supplementation supports

higher metabolic activity and pinhead formation. However, no treatment yielded mature mushrooms, which highlights that nutrient enrichment alone does not suffice for complete development (Table 4). Similar challenges have been reported by Li *et al*. (2023), who concluded that successful fruiting in *Morchella* requires not only optimal nutrition but also precise control of microclimatic factors, including humidity, temperature, photoperiod, and CO_2 levels. The failure of maturation across treatments suggests that factors such as low humidity, temperature fluctuations, or inadequate light exposure may have disrupted the critical transition from primordia to mature fruiting bodies.

Table 4. Evaluation of different compost substrates for mushroom cultivation.

Treatments	Spawn Run (days)	Pinhead Formation (days)	No. of Fruiting Bodies	Total Yield (g)
50% Soil & 50% Wheat straw	35 b	50 b	5 b	2 b
50% Soil and 50% Sawdust	40 a	55 a	7 ab	3 b
50% Soil, 25% Organic matter, 25% Peat moss	38 a	53 ab	8 a	4.2 a

CONCLUSION

The findings of this study reaffirm that PDA is the most suitable culture medium for *M. esculenta* mycelial development, and that barley grain is the most effective substrate for spawn production. Although substrate treatments with nutrient supplementation promoted better fruiting initiation, the lack of mature fruit bodies across all treatments indicates that further refinement of both nutrient composition and environmental controls is essential.

FUTURE DIRECTIONS

Future studies should explore integrated approaches involving precise environmental regulation, enhanced nutrient formulations (including micronutrient supplementation), and possibly mycorrhizal associations, which have been shown to influence morel development in natural settings.

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Tariq Mukhtar	:	Conceptualized the idea for the study and supervised the research.
Gulshan Irshad	:	Provided support at the URF.
Muhammad Tariq	:	Designed the methodology and guided in analyzing the data.
Syed Z. Ali	:	Helped in data collection.
Amar Mehmood	:	Helped in data collection.
Kachu Z. Haider	:	Helped in data collection.
Rahim U. Din	:	Helped in data collection.
Md. Arshad Ali	:	Provided technical assistance and refined the manuscript.
Khim P. Chong	:	Provided technical assistance and refined the manuscript.